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[001] Device for raising or cultivating cells
in a container-like receptacle

[002]

[003] The invention relates to a device for raising or cultivating cells in a container-like receptacle which comprises a base and at least one lid.

[004]

[005] For laboratory requirements, it is known to raise or cultivate cell cultures in a shallow receptacle or dish, the cells simply being placed in the receptacle and culture medium being added. A lid is then placed on the receptacle.

[006] A disadvantage is that this method can only be used for small quantities. In particular, the known system is not suitable for cultivating or raising cells in batches. Moreover, it is impossible to achieve *in vivo* conditions, and sterility is not guaranteed.

[007] An alternative to this was to use a closed system which comprised a receptacle with a lid or closure piece and which permitted sterility. However, a disadvantage of this was that removing the cell culture was very laborious and time-consuming.

[008] It is therefore an object of the present invention to improve a device of the type mentioned at the outset in such a way that it can be used in a highly versatile manner for raising or cultivating cells, particularly in large-scale operations, the aim being to achieve as far as possible *in vivo* conditions and sterility. Moreover, after they have been cultivated, the cell cultures should be able to be removed from the receptacle without great effort and without being dam-

aged.

[009]

[010] According to the invention, this object is achieved by the features in the characterizing part of claim 1.

[011] In the device according to the invention, the cells in the receptacle are no longer "left to themselves", and instead an active process takes place in practice. On the one hand, culture medium can be introduced continuously or intermittently, and, on the other hand, it is possible for the cell cultures forming to be acted upon with pressure. This pressure actuation can even be effected with alternating pressure in order to simulate natural conditions as far as possible.

[012] The cells can be raised or cultivated as separate cell cultures. In the same way they can also be formed on structures in order to produce implants.

[013] In the receptacle according to the invention, the cells can in this way also be exposed to shearing forces or pressure forces. With the device according to the invention, a wide variety of cell cultures can be raised or cultivated in a highly versatile manner:

[014] The receptacle itself can also be provided for this purpose variously with one or two lids. Likewise, a common connector bore can be provided for the introduction of culture medium and for its return. Of course, separate connector bores are also possible, in which case parallel flows or through-flows are possible depending on the arrangement of the connector bores.

- [015] By virtue of the configuration according to the invention with one or two lids or a lid and base, and between them a receptacle which can simply be a cylinder open at the top and bottom, the cell cultures can easily be removed from the receptacle, after they have been treated or raised, without great effort and without damaging them.
- [016] Since according to the invention the cells are raised or cultivated on the lower lid or base or also under the upper lid, e.g. on a frame connected to the upper lid, the cell cultures can be easily removed from the receptacle once they are ready. In any event, good accessibility is afforded by the removable lids or base.
- [017] For pressurization, the receptacle, e.g. the circumferential wall of the cylindrical receptacle, can also be made elastic.
- [018] The connector bore or connector bores can be arranged in one lid or, if two lids are present, in both lids. Likewise, it is also possible to form the connector bores in the cylindrical middle part. The number and arrangement of the connector bores depends on the application and on the cells which are to be raised or cultivated.
- [019] For a tight connection between the lid or lids and the receptacle, clamp connections, sealing rings or threaded connections with internal and external threads can be provided.
- [020] Very good sealing conditions and thus pressure conditions are obtained if the lid or lids are provided with extension rings which then sealingly enclose the cylindrical middle part of the receptacle from the outside.

- [021] If the device according to the invention is to be subjected to a rolling or turning movement, tensioning rings can be fitted laterally onto the device, which tensioning rings grip the lid or lids and the receptacle, and a turning or rolling means can then be applied to them.
- [022] A wide variety of pressurizing means can be used to subject the interior of the receptacle forming the cell culture chamber to pressure. Suitable for this purpose are, for example, cylinder/piston units which can also operate in pulsed mode for alternating pressure loads.
- [023] If necessary, the receptacle can also be designed as a two-chamber system so that two different cells or two identical cells can be cultivated or raised separate from one another.
- [024] In this case, it is advantageous if the lid of the receptacle is provided with a suspension means on which a platform for receiving cells is arranged. In this way, one cell type is raised on the platform, while another cell type can be cultivated on the base of the receptacle.
- [025] If necessary, the receptacle or the cylindrical circumferential wall of the receptacle can be made porous or gas-permeable, so that in this way too culture medium and/or a gaseous medium, for example air or oxygen, can be delivered from this side.
- [026]
- [027] Advantageous developments and configurations will be evident from the remaining dependent claims and from the illustrative embodiments described below with reference to the drawing, in

which:

- [028] Fig. 1 shows a cross section through a first embodiment of a device with a receptacle and a lid;
- [029] Fig. 2 shows a cross section through a device with a cylindrical middle part and an upper lid and lower lid;
- [030] Fig. 3 shows a similar configuration to the one in Fig. 2, with an inlet connector and an outlet connector being arranged in the upper lid;
- [031] Fig. 4 shows a further embodiment of the device according to the invention;
- [032] Fig. 5 shows a device according to the invention with an upper lid and a lower lid, each with external thread, and a cylindrical middle part with internal thread;
- [033] Fig. 6 shows an embodiment with extension rings on an upper lid and a lower lid;
- [034] Fig. 7 shows a similar configuration to the one in Fig. 6, with a clamp connection;
- [035] Fig. 8 shows a similar configuration to the one in Figures 6 and 7, with threaded connections;
- [036] Fig. 9 shows an embodiment with two lateral tensioning rings;
- [037] Fig. 10 shows an embodiment similar to the embodiment in Fig.

9, with a pressurizing means;

[038] Fig. 11 shows an embodiment with a suspension means in the upper lid, with a cylindrical middle part and a lower lid;

[039] Fig. 12 shows a similar configuration to the one in Fig. 11, in somewhat simpler form with just an upper lid;

[040] Fig. 13 shows a configuration with a pressure force generated by magnetic forces;

[041] Figures 13a, 13b and 13c show different pressure disk profiles;

[042] Fig. 13d shows a mineral matrix for bone replacement as support structure with two pressure disks;

[043] Fig. 14 shows a configuration with expandable elements for generating pressure forces;

[044] Fig. 15 shows a configuration with hydraulic or pneumatic elements for generating pressure forces;

[045] Fig. 16 shows a similar configuration to the one in Fig. 15;

[046] Fig. 17 shows a further configuration similar to those in Figures 15 and 16;

[047] Fig. 18 shows a configuration with a gas-permeable membrane as base;

[048] Fig. 19 shows a configuration with a three-chamber system;

[049] Fig. 20 shows a similar configuration to the one in Fig. 19.

[050]

[051] According to the illustrative embodiment in Fig. 1, which shows the basic configuration of the device according to the invention, a receptacle 1 is provided which has an external thread 2. A lid 3 with an internal thread 4 is screwed onto the receptacle 1, and a sealing ring 5 forms a pressure-tight closure of a cell culture chamber 6 for cells 7 provided in the interior of the receptacle 1.

[052] The lid 3 is provided with an inlet connector bore 8 and an outlet connector bore 9 in order to introduce culture medium and if appropriate also oxygen into the cell culture chamber 6 via corresponding lines or tubes.

[053] For easier handling, a tray 10 can be inserted into the receptacle 1 so as to make it easier to insert and remove the cells 7 which are to be cultivated or raised. For this purpose, beads, flanges 11 or the like which are provided in the upper area of the tray 10 can also serve for easier removal and easier insertion. The tray 10 shown only in Fig. 1 can of course also be provided in the same way or in a similar way in the other illustrative embodiments.

[054] Fig. 2 shows a configuration where the receptacle 1 forms a cylindrical middle part which can be closed with the upper lid 3 in the same way as in the illustrative embodiment according to Fig. 1. Provided on the underside there is a further, lower lid 12 which forms the base of the receptacle and which likewise has an internal thread 4 which interacts with

the external thread 2 of the middle part, said middle part in this case being provided with two external threads 2, unless one continuous thread is present. As will also be apparent from Fig. 2, inlet and outlet connector bores 8 and 9 are provided in both lids 3 and 12.

- [055] Fig. 3 shows a similar configuration to the one in Fig. 2, with an inlet connector bore 8 and an outlet connector bore 9 being arranged only in the upper lid 3. In the same way as in the illustrative embodiment according to Fig. 2, sealing rings 5 are provided on both ends of the cylindrical middle part of the receptacle 1.
- [056] Fig. 4 shows an embodiment in which the cylindrical middle part as receptacle 1 is provided with an internal thread 2' which cooperates with an external thread 4' of the upper lid 3 and of the lower lid 12. Here too, sealing rings 5 are provided on both ends of the receptacle 1. In this case, an inlet connector bore 8 is provided in the upper lid 3, and an outlet connector bore 9 is provided in the lower lid 12.
- [057] Fig. 5 shows a similar configuration to Fig. 4. The main difference is simply that the inlet connector bore 8 and the outlet connector bore 9 are arranged opposite one another in the cylindrical middle part of the receptacle 1.
- [058] Fig. 6 shows an embodiment with a cylindrical middle part as receptacle 1, an upper lid 3, and a lower lid 12. Both lids 3 and 12 each have an external thread 4' which cooperates with internal threads 2' of the receptacle 1. In addition, both lids 3 and 12 are provided with radial extensions 13 from whose outer ends extension rings 14 extend axially into the

receptacle 1, parallel to the longitudinal axis of the receptacle 1. The extension rings 14 enclose the outer wall of the receptacle 1 and, together with additional sealing rings 5', constitute a pressure-tight closure for the cell culture chamber 6. The upper lid 3 is provided with a common connector bore 8, 9 for the introduction and withdrawal of culture medium.

[059] Fig. 7 shows a similar embodiment to the one in Fig. 6. The main difference is simply that, instead of a threaded connection 2', 4', a pressure-tight closure of the cell culture chamber 6 is created by clamp connections between the extension rings 14 and the outside wall of the receptacle 1.

[060] Fig. 8 shows an embodiment similar to those in Figures 6 and 7. The main difference here is that the threaded connection between the receptacle 1 and the lids 3 and 12 is formed by internal threads 4' in the extension rings 14, these cooperating with external threads 2' in the receptacle 1.

[061] Fig. 9 shows an embodiment with a receptacle 1 and an upper lid 3, similar to the embodiment according to Fig. 8, but instead of a common connector bore 8, 9 for the introduction and withdrawal of culture medium, an inlet connector bore 8 and an outlet connector bore 9 are arranged in the circumferential wall of the receptacle 1. In addition, Fig. 9 shows two lateral tensioning rings 15 which are pushed round the container-like receptacle 1 and the lid 3 in the arrow direction so that the unit consisting of receptacle 1 and lid 3 can be turned or rolled in arrow direction 16 about the transverse axis with the aid of a turning or rolling means (not shown).

- [062] Fig. 10 shows a similar configuration to the one in Fig. 9. In this case, a separate cell culture chamber 6 is formed in the interior of the receptacle 1. Instead of a cell culture chamber 6, it is also possible to provide a structure on which the cells 7 are raised. The separate cell culture chamber 6 or the structure is in this case pressurized via a pressurizing means 17 in the form of a cylinder/piston unit.
- [063] The inlet connector bore 8, which can be shut off at the entry point by a check valve 19, opens into a piston chamber 18 of the cylinder/piston unit 17. The culture medium introduced through the inlet connector bore 8 is pressurized by a piston 20 of the cylinder/piston unit 17, and this pressure continues into the interior of the receptacle 1. Culture medium is withdrawn via an outlet connector bore 9 on the side of the receptacle 1 directed away from the inlet. When the interior of the receptacle 1 is to be subjected to an overpressure, which may if appropriate be alternating, the return flow of culture medium will in this case be constricted or the outlet connector bore 9 correspondingly shut off.
- [064] Instead of supplying culture medium via the inlet connector bore 8, a separate bore can also be provided for this in one of the two lids 3 or 12 or in the circumferential wall of the receptacle 1. In this case, it is also possible to use gas, e.g. air, to pressurize the interior of the receptacle 1 and thus the cell culture chamber 6.
- [065] Fig. 11 shows an embodiment with an upper lid 3 and a lower lid 12 and a cylindrical middle part of a receptacle 1. In this case, the upper lid 3 is provided with a suspension

means in the form of several rods 21 which are distributed about the circumference and which extend, parallel to the longitudinal axis of the receptacle 1, into the interior of the receptacle 1. Secured at the lower end of the rods 21 there is a platform 22 on which the cells 7 to be cultivated or raised are arranged. The inlet connector bore 8 and the outlet connector bore 9 can each be arranged in the circumferential wall of the receptacle 1. Of course, it is also possible to arrange them in one of the two lids 3 or 12, as is indicated by broken lines. In this case too, separate connector bores are of course also possible for inlet and outlet.

- [066] The advantage of the embodiment with the suspension means formed by the rods 21 is that in this way the cells 7 are easier to insert into the receptacle 1 and remove therefrom.
- [067] If necessary, the connection of the rods 21 to the platform 22 can be made detachable. Detachability can be achieved, for example, by a clip connection, which also provides for easier handling of the device.
- [068] Fig. 12 shows an embodiment which is similar to the embodiment shown in Fig. 11. As will be noted, it only has an upper lid 3 and a receptacle 1 with base 23, as is also present in the other embodiments with just one lid 3. The platform 22 in this case is again connected to the upper lid 3 via rods 21.
- [069] A further advantage of the embodiments according to Figures 11 and 12 is that the receptacle base 23 or inner side of the lid 12 provides an additional possibility for raising or cultivating cells 7. In this way, a two-chamber system for cul-

tivation of two cell cultures is created.

- [070] Instead of inlet connector bores 8 and outlet connector bores 9 for culture medium, culture medium can of course also be introduced continuously into the receptacle 1, and the inlet connector bores 8 and outlet connector bores 9 then serve only for oxygen supply.
- [071] Alternatively, it is also possible to provide separate connector bores for oxygen and culture medium.
- [072] The platform 22 can be designed as a solid unit. Alternatively, it is also possible for this purpose to provide a membrane, e.g. a porous membrane, which allows oxygen to pass through.
- [073] Figures 13 through 20 show further configurations of the invention, the basic structure of the device with receptacle 1 and both lids 3, 12 and/or base 23 having been retained, for which reason, to keep matters simple, only the relevant reference numbers have been repeated in the following description of these figures.
- [074] Fig. 13 shows a receptacle 1 in which a magnetic means 24, e.g. a magnet coil through which current flows, is incorporated in the area of the upper lid 3. Under the magnetic means 24 there is a magnetizable pressure disk 25 which is connected elastically to the receptacle 1 in a manner not shown in detail.
- [075] Through a movement of the pressure disk 25 caused by activation of the magnetic means 24, generated by alternate current

directions for example, an internal pressure load is exerted on the cells 7.

- [076] Fig. 13a shows a plan view of the profile of a pressure disk 25, small openings 26 being provided so that culture medium located in the inside of the receptacle 1 can pass through.
- [077] Figures 13b and 13c show alternative pressure disks 25 in the form of a mesh structure or grid structure so that culture medium can pass through.
- [078] Of course, the magnet coil acting as magnetic means 24 can also be arranged outside the lid 3, above the latter. In this configuration, the lid 3 of course has to be made of nonmagnetizable material, e.g. plastic. In this case, suitably large magnetic means 24 can be provided and correspondingly high pressure forces generated.
- [079] Figure 13d shows a configuration of an implant, cartilage profiles 27 being arranged on a mineral matrix for bone replacement as support structure 27a. Here, two support structures are provided which are arranged one above the other and on each of which a pressure disk 25 is arranged. The mineral matrix can, for example, be a bone structure, e.g. of calcium phosphate.
- [080] The mineral matrix can also have other profiles as are required for implants, e.g. joint structures. It is also possible, of course, to deviate from the circular shape. The same also applies in principle to the receptacle 1.
- [081] Fig. 14 shows a configuration with expandable elements 28

which axially displace a plate 29 arranged movably in the receptacle 1 or the lid 3, in the same way as the pressure disks 25, and thus can exert alternating pressure forces on the cells 7. The expandable elements used can, for example, be shape-memory metals or plastics which deform and then return to the original shape. Thus, for example, there are also plastics which can expand through electrical change. Elements with shape-memory function react, for example, to certain temperatures or to ultrasound and in this way alter their state, thus generating a movement of the plate 30. Spring devices are also possible, as are motors with accumulators or batteries.

[082] Fig. 15 likewise shows internal pressurization of cells 7 by a hydraulic or pneumatic means 30 arranged in the receptacle 1 or in the lid 3. As will be noted, the means 30 has a movable film, plate or membrane 31, behind which a hydraulic liquid or a gas acting as fluid is located. The hydraulic liquid or the gaseous medium is subjected to alternating pressure by a pressurizing means P (not shown in detail), as a result of which alternating pressure loads are exerted on the cells 7. Instead of an elastic plate or membrane 31, a balloon can also be used, if required, in order to obtain the possibility of greater variation. For example, all the walls of the receptacle can be covered on the inner side by such a bag or balloon, in which case the implant or cell cultures are located in the inside. In this way, an alternating pressure load is exerted all round from outside.

[083] Fig. 16 shows a similar configuration in which the cells 7 lie in a gel 32.

- [084] Fig. 17 likewise shows a similar configuration to the one in Fig. 16, the sealing between the upper lid 3 and the lower lid 12 being obtained by sealing rings 33 and 34.
- [085] Fig. 18 shows a configuration in which the base of the receptacle is formed by a gas-permeable membrane 35, e.g. of PTFE or silicone. The important thing is that oxygen can pass into the receptacle 1 through the membrane 35. In this configuration too, an implant growing in the interior of the receptacle can be easily removed. Such a configuration is suitable, for example, for growing epidermis 36, i.e. the top layer of the skin, on the membrane 35. The cells are supplied with air through the gas-permeable membrane 35. The aim here is to have the cells grow from the underside upward. In addition, a dermis 37 is then applied on top, the cells being arranged or cast in a collagen structure or in fibrin. At the top, above the dermis 37, there is a clean area 38 in which air, oxygen and/or carbon dioxide is introduced. Pressurization is also possible here.
- [086] In this way, a skin structure is obtained as exists in reality. If so required, various other cells can also be added, for example endothelial cells in order to achieve vascularization on the top face.
- [087] To achieve sterility and protection for the membrane 35, a covering structure 39 can, if required, be fitted tightly or screwed on at the bottom.
- [088] On the inside, the receptacle can be provided with a peel-off film 40 for transplantations. On completion of the cell cultivation, the covering structure 39 is in this case removed

and the film 40 is then peeled off like a plaster. In this way, the implant can be easily removed and then used. The dermis 37 then lies in the correct position on the wound and, when the film is peeled off, the epidermis 36 lies on top.

[089] Instead of a covering structure 39, a chamber with lateral openings can also be provided, in which case a sterile filter is fitted.

[090] Fig. 19 shows a similar configuration to the one in Fig. 18. Here, by contrast, a multi-chamber system is formed, with an upper chamber 41, a middle chamber 42 and a lower chamber 43. Separate inlets and outlets can be provided for all three chambers 41, 42, 43. Thus, for example, the epidermis 36 can be arranged in the chamber 41, the dermis 38 can be arranged in the chamber 42, and the lower chamber 43 is used for the supply of air or oxygen. Culture medium can be introduced into the chamber 41 in a first step. Then, in a second phase after the cells 7 have grown, if differentiation is desired, oxygen or air can be introduced into the chamber 41 so that the epidermis cells 36 acquire a dry environment and are then able to dry out and keratinize. In this way, the upper layer of skin is then formed. If one then wishes to implant the whole arrangement, the receptacle can be easily opened and the implant removed.

[091] If the cells 7 arranged in the chamber 42 require a support structure, a porous support or membrane 44 can be provided on the underside and constitutes the division between the chamber 42 and the chamber 43.